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ANTIMALARIAL ACTIVITY OF 80% METHANOLIC EXTRACT OF *BRASSICA NIGRA* (L.) KOCH. (*BRASSICACEAE*) SEEDS AGAINST *PLASMODIUM BERGHEI* INFECTION IN MICE

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LISTS OF ABBREVIATIONS AND ACRONYMS

ACTs	Artemisinin-Based Combination Therapies
ADRs	Adverse Drug Reactions
ANKA	Antwerp-Kasapa
ANOVA	Analysis Of Variance
DNA	Deoxyribonucleic Acid
HIV	Human Immunodeficiency Virus
HSD	Honestly Significant Differences
IP	Intra-Peritoneal
IRBCs	Infected Red Blood Cells
MST	Mean Survival Time
RBCs	Red Blood Cells
RDTs	Rapid Diagnostic Tests
SEM	Standard Error of the Mean
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organization

ABSTRACT

Background: Resistances to currently available drugs and insecticides, significant drug toxicities and costs and lack of vaccines currently complicated the treatment of malaria. A continued search for safe, effective and affordable plant-based antimalarial agents, as evident from history, thus becomes crucial and vital in the face of these difficulties.

Objective: To evaluate the antimalarial activity of 80% methanolic extract of the seeds of *Brassica nigra* against *Plasmodium berghei* infection in mice.

Method: The seed of *Brassica nigra* was extracted by 80% methanol using maceration method. Chloroquine sensitive *Plasmodium berghei* (ANKA strain) was used to test the antimalarial activity of the extract. In suppressive and prophylactic models, Swiss albino mice of either sex were randomly grouped into five groups of five mice each. Group I mice were treated with the vehicle (distilled water, 10 ml/kg), group II, III and IV were treated with 100, 200, and 400 mg/kg of the extract, respectively and the last group (V) mice were treated with chloroquine (10 mg/kg). The level of parasitemia, survival time and variation in weight of mice were used to determine the antimalarial activity of the extract.

Results: Chemosuppressive activities produced by the extract of the seeds of *Brassica nigra* were 21.88%, 50.00% ($P < 0.01$) and 53.13% ($P < 0.01$) suppression, while the chemoprophylactic activities were 17.42%, 21.21% and 53.79% ($P < 0.05$) suppression at 100, 200 and 400 mg/kg of the extract, respectively as compared to the negative control. The extract treated mice were significantly ($P < 0.05$) lived longer and gained weight as compared to negative control at 200 and 400 mg/kg of the extract in suppressive test.

Conclusion: From this study, it can be concluded that the seed of *Brassica nigra* showed good chemosuppressive and moderate chemoprophylactic activities and the plant may contain biologically active principles which are relevant in the treatment and prophylaxis of malaria, thus supporting further studies of the plant for its active components.

Key words*:* Antimalarial activity, *Brassica nigra, In vivo,* Malaria, Mice, *P. berghei.*

1. INTRODUCTION

1.1. Background Information

1.1.1. History of Malaria

Malaria is one of the most ancient and prominent disease. Chinese traditional medical books recorded the symptom consistent with malaria which was spread by the biting of insects that could be treated with *Artemisia annua*, which contains artemisinin, a modern effective antimalarial drug, as early as 2700 BC. In 500 BC, the great impact of the disease on city-states and its symptoms was described by Hippocrates. In the 17th century, Spanish Jesuits brought *Cinchona* bark, which contains quinine that effectively treated those patients encountered with malaria fever, from Peruvian Indian to Europe [1].

In ancient Rome malaria was prevalent in stagnant waters of marshes. The ancients blamed that the foul air evaporating from such areas were the origin of the disease. Hence, the name is derived from the Italian word, "*mal aria*," which means *bad air*. However, in 1880, the actual etiologic agent of malaria, single-celled *Plasmodium* parasite, was discovered by Alphonse Laveran. Female *Anopheles* mosquitoes, which transmit the disease, were identified by Ronald Ross in 1898 [2].

1.1.2. Epidemiology of Malaria

Malaria is a serious hazard to humanity and the major cause of mortality and morbidity in the malaria-endemic countries. Even though the distribution of the disease is substantially varied, sub-Saharan Africa, Asia and Central and Latin America are the most affected regions, respectively, in the world. It is estimated that about 40% of the world population reside in such regions [3].

According to World Health Organization (WHO) world malaria report in 2014, about 50% of populations in the world live in malaria risk areas. About 198 million cases of malaria and 584,000 malarial deaths have been estimated worldwide in 2013. More than 80% and 90% of these cases and deaths, respectively, were in Africa. However, malarial cases and its related

deaths have been reduced from 50% to 75% over the past decade. The death rates of malaria have decreased by 47% and 54% globally and in Africa, respectively, between 2000 and 2013. However, emergence of resistance to drugs and insecticides continues to pose a major threat, if left unresolved could trigger an upsurge in deaths of malaria [3].

In 2015, if the annual rate of decrease over the past 13 years is maintained, malaria mortality rates are projected to decrease by 55% and 62% globally and in Africa, respectively, and in children under 5 years of age are projected to decrease by 61% and 67% globally and in Africa, respectively [3].

The poorest and most vulnerable communities are highly burdened with malaria. Tropics such as Africa in general and sub-Saharan Africa in particular, homeland of the most dangerously malaria-transmitting mosquito and parasite of malaria, are the worst-affected regions in the world. More than 80% of African populations are at risk of malaria. Children under 5 years of age and pregnant women are the most risky population. In 2013, about 163 million cases of malaria have been estimated in Africa and 78% of them were in sub-Saharan Africa. About 528,000 malaria deaths have been reported in Africa and 83% of them were children under 5 years. It also has devastating economic consequences as it drains as much as 2% of the gross domestic product of countries in sub-Saharan Africa. It also has devastating economic consequences as it drains as much as 2% of the gross domestic product of these countries [3].

Malaria continues to be a major public health concern in Ethiopia. About 75% of its land is malarious with 68% of its population reside in such malaria-risky areas. The pattern of the disease is seasonal in most parts of the country. Altitude, rainfall and population movement have great impact on the transmission and prevalence of the disease. Altitudes below 2000 meters above sea level are considered to be malaria-endemic areas in the country [4].

Currently, the burden of the disease in Ethiopia have been declined by more than 50% nationwide compared to the pre-intervention period. About 2,645,454 clinically confirmed cases and 358 inpatient deaths of malaria have been reported nationwide in 2013 [3]. Despite these important gains, the transmission is highly diversified across the different regions and places of the country [4].

1.1.3. Causes and Transmission of Malaria

Malaria is caused by obligate intracellular protozoa parasite of the genus *Plasmodium*, belonging to the parasitic phylum *Apicomplexa* and family *Plasmodidae*. More than 200 species of the genus have been identified that are parasitic to reptiles, birds, and mammals. Only five species can infect humans. *Plasmodium falciparum* (the most dangerous), *P. vivax*, *P. malariae* and *P. ovale* are human malaria species. Recently, *P. knowlesi*, the monkey malaria parasite in Southeast Asia, also causes diseases in humans. In Ethiopia, *P. falciparum* and *P. vivax* are the dominant species of the malaria parasite [4, 5, 6].

The disease is mainly spread through the bite of an infected female mosquito of the genus *Anopheles* (family *Culicidae*). Of more than 480 species identified, only about 50 species have been implicated in the transmission of the disease. The infected mosquitoes themselves remain infectious until death, and untreated or inadequately treated individuals can be sources of infection to mosquitoes for a period of 2–3 years. Infected blood transfusion, needle-stick injury, sharing of needles, congenitally and organ transplantation can also contract the disease even though the incubation period of the parasite is often short [5].

1.1.4. Biology and Pathogenesis of Malaria

All *Plasmodium* species have multistage, complex and nearly identical life cycles, shown in Figure 1. Infection starts when sporozoites are injected by an infected mosquito during blood meal and carried around the body to the liver where merozoites are produced via asexual reproduction. Some portions of *P. vivax* and *P. ovale* form hypnozoites typically remain dormant in the liver which cause relapses and pose a significant obstacle for the eradication of the disease. From 10,000 to 30,000 daughter merozoites might be produced from a single sporozoite. Eventually, theses merozoites are discharged into the bloodstream after bursting of the swollen infected liver cells [5, 7].

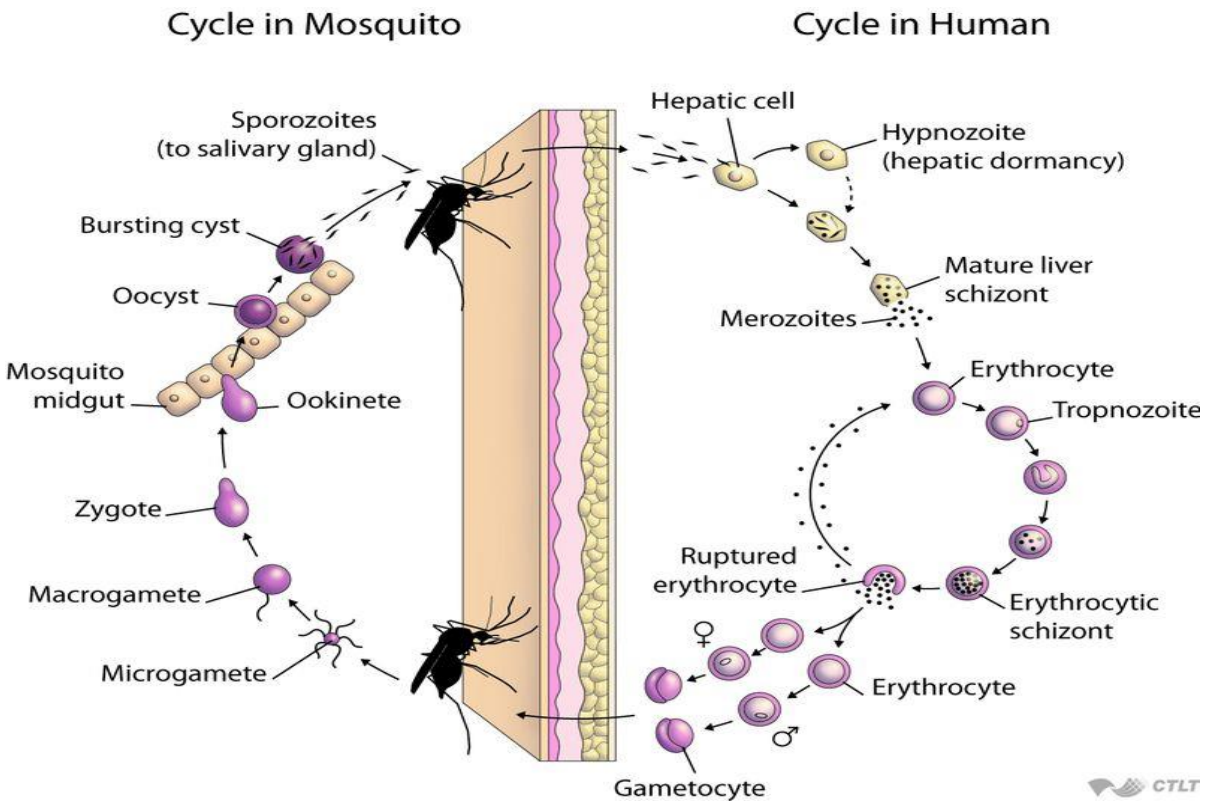

Figure 1. The Life Cycles of the Malaria Parasites [8].

Once merozoites leave the liver, they invade red blood cells (RBCs) and multiply 6- to 20-fold every 24–72 h by second asexual multiplication. The infected RBCs are lysed by the merozoites (6–30 daughter merozoites released per infected RBCs), which subsequently invade other RBCs, starting a new cycle of schizogony. This phenomenon is repeated indefinitely and is responsible for the disease, malaria. Its clinical manifestations and the sources of infection to mosquitoes are mainly due to such schizont rupture, and destruction of RBCs and the host's reaction to this process [5, 7].

After several cycles, some of the merozoites develop into longer-lived sexual forms of male (micro-) and female (macro-) gametocytes. These gametocytes are then taken up by a mosquito upon blood feeding. Within the mosquito midgut, they are converted into gametes and fertilized into ookinete and then multiplied asexually into oocyst and finally burst into sporozoites. Sporozoites migrate to the salivary glands of a mosquito and are injected when the mosquito feeds on a new host [9].

Only the blood stage of the parasite causes manifestations of uncomplicated and severe malaria. The growing malaria parasite progressively degrades and consumes its intracellular proteins particularly hemoglobin after infecting RBCs. The parasite also alters its membrane by changing its transport properties, exposing cryptic surface antigens, and inserting new parasite-derived proteins. The RBC becomes more irregular in shape, more antigenic and less deformable. This alteration leads to the adherence of infected RBCs to endothelial cells, uninfected and infected RBCs called cytoadherence, rosetting and agglutination, respectively. These adherence processes involve a multistep interaction between parasite derived molecules expressed on the RBC surface and receptor molecules present on the surface of endothelial cells or other RBCs. These processes are central for the pathogenesis of *P. falciparum* malaria and hence results in the sequestration of infected RBCs in vital organs including brain. In addition, sequestered parasites mask themselves from the principal host defense mechanism of splenetic processing and filtration [5].

Severe infection also reduced deformability of the normal RBCs, which compromises their passage through the partially obstructed blood vessels and shortens their survival. Cerebral malaria, pulmonary edema &/or acute respiratory distress syndrome, renal failure, severe anemia, hypoglycaemia, metabolic acidosis, intravascular coagulation &/or bleeding, convulsions, and circulatory collapse are among the common manifestations of severe malaria. Therefore, it is a multisystem disorder. Severe malaria in children, pregnant women and in patients with HIV co-infection has increased morbidity and mortality [5, 6, 10].

1.1.5. Diagnosis of Malaria

Early diagnosis and prompt treatment are vital to minimize the morbidity and mortality of malaria. Malaria cannot be diagnosed clinically as its signs and symptoms can mimic other tropical viral infections; hence, the disease must be confirmed by laboratory diagnosis. The simplest and gold standard method for diagnosing of malaria is light microscope. Both thick and thin stained blood smears are prepared and looked under the microscope. Although there are many techniques for staining of blood films, Giemsa at pH 7.2 is the preferred one [5]. Currently, there are rapid diagnostic tests (RDTs) for malaria. RDTs offer a simple, sensitive, specific and quick supplement to microscopy especially for *P. falciparum* detection [11].

1.1.6. Treatment of Malaria

Malaria is easily preventable and treatable if the following measures are taken which can include proper diagnostic tools, availability of antimalarial drugs, bed nets and insecticides and vaccines. Malaria vectors have been eliminated from several areas by using bed nets impregnated with insecticides and indoor residual spraying techniques [12].

Currently antimalarial drugs can be used as prophylaxis, treatment of falciparum and treatment of non-falciparum malaria. Drugs used for the treatment of malaria can be categorized into five groups as antifolates (pyrimethamine, proguanil, sulfadoxine, sulfadiazine), sesquiterpenes (artemisinin, dihydroartemisinin, artesunate, artemether, arteether), quinolones (quinine, chloroquine, mefloquine, amodiaquine, primaquine, halofantrine), naphtoquinones (atovaquine) and antibiotics (tetracyclines, clindamycin) [13].

Different factors should be considered during the selection of antimalarial drugs. Generally, the choice of antimalarial drug depends on the diagnosis of each *Plasmodium* species, the level of parasitemia, type and level of drug resistance in the region where the infection was acquired, the stage of infection, and the availability of antimalarials and other factors [14].

The first line treatment for uncomplicated falciparum malaria is artemisinin-based combination therapies (ACT) including artemether plus lumefantrine, artesunate plus amodiaquine, artesunate plus mefloquine and artesunate plus sulfadoxine-pyrimethamine while for uncomplicated non-falciparum malaria are treated by chloroquine or ACT. Severe malarias are treated by intravenous artesunate (drug of choice), artemether, quinine or quinidine. Primaquine, malarone, aminoquinine, mefloquine, chloroquine and doxycycline are used for chemoprophylaxix [15, 16, 17].

Currently the most effective antimalarial therapies are ACTs and with vector control, they have led to a significant decline in the burden of the disease. To maximize their effectiveness and to protect them from the development of resistance, they are combined with other drugs with different mechanisms of action and longer half-lives. However, resistance to most of ACTs will probably lead to a global epidemic outbreak of malaria [16].

However, the main drawbacks associated with antimalarial drugs are the development of single or multiple drug resistance and the non-specific targeting to intracellular parasites which results in high dose requirements and subsequent intolerable toxicity that provides a new vision to apply novel approaches in disease treatment. Hence, various lead molecules are needed to target the parasite at its different life cycles. The potential new antimalarials under phase II and III development include lopinavir/ritonavir; zidovudine; lamivudine; OZ439; KAE609; NITD609; GNF156; *Argemone mexicana*; etc [18].

1.1.7. Vaccines for Malaria

Although malaria can be effectively controlled in high-risk endemic areas, the complete eradication of the parasite is still a considerable challenge. Malaria can only be eliminated from the world by the reduction of poverty or by the development of effective and affordable vaccine. A safe, effective and affordable malaria vaccine would create a powerful public health benefit by removing the gap left by other interventions. Therefore, a malaria vaccine can add an important, complementary tool to the existing malaria interventions [19, 20].

The three main focus areas currently under investigation for vaccine developments are pre-erythrocytic, erythrocytic and transmission life cycles of the parasite. More than one and two dozen active malaria vaccine candidates are under in preclinical and clinical developments, respectively. The majority of them targeted *falciparum* malaria [21].

There is no vaccine against malaria has been approved yet. But the only vaccine candidate in large-scale, late-stage testing, is the *RTS,S* candidate. The latest phase III clinical trial showed that the vaccine reduced about 50% clinical malaria cases in African children and infants. It has an acceptable safety profile. It may be available in late 2015 [19].

However, many market and scientific challenges exist in malaria vaccine development. The parasites' complex life cycles, inadequate funding, too little cooperation among scientists and funding agencies, limited private-sector involvement and mixed levels of interest from developing countries pose a significant barrier to the progress of malaria vaccine development [21].

1.2. Statement of the Problem

Resistances of mosquitoes to insecticides and of parasites to antimalarial drugs especially to the current most effective and newest artemisinin have led to an increase in complicated malaria and complicate the eradication of the disease as well as the resurgence of malaria. While there is much need and there is currently no alternative to the precious artemisinin derivatives, the antimalarial drug development pipeline remains sadly thin with little chemical diversity [22].

Preventive and treatment strategies of malaria are not only continuously hampered by the issues of resistance but also considerable costs and logistical problems especially in poor malarious countries as well as a challenge of having effective vaccines create a great and urgent concern for the treatment of the disease [23].

Additionally, many antimalarial drugs in use today have high toxicity and low therapeutic margin of indices that exposes patients' additional harm and health expenditure. Especially genetic abnormalities among patients play a significant role in the incidence and severity of these undesired reactions to many antimalarial drugs [24].

The efficacy, safety and quality of most medicinal plant products in traditional medicine are not validated and standardized. In addition, their active constituents are not identified. These products can be easily adulterated with other plant materials and contaminated with foreign substances. Medicinal plants used in traditional medicine should, therefore, be studied for their safety and efficacy [25, 26, 27].

Due to their long usage in the treatment of diseases according to knowledge accumulated over centuries, plants commonly used in traditional medicine are generally assumed to be safe. However, recent scientific findings have shown that many plants used in traditional medicine are potentially toxic, mutagenic and carcinogenic. Their genetic, reproductive and developmental toxicities should, therefore, be assessed. Their interactions with modern drugs are also a great concern [28, 29].

1.3. Literature Reviews

1.3.1. Antimalarial Drug Resistance

Drug resistance in malaria can be defined as "the ability of a parasite strain to live and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended within the limits of tolerance of the patient" [30].

The emergence of antimalarial drug resistance is a recent phenomenon even if longer history of usage of such drugs. Chloroquine-resistant strains of *P. falciparum* malaria initially appeared in Thailand in 1957, and then spread through South and Southeast Asia. By the 1970s, they were being seen in sub-Saharan Africa and South America. A worldwide increase in malaria-related mortality, particularly in sub-Saharan Africa, was due to the rise in chloroquine resistance. In addition, a common consequence of such drugs resistance, which results in repeated infection, is anemia [5, 31].

Drug monotherapies against malaria have established effective, even if transient, owing to the inevitable evolution of resistant parasites. Hence, drug combination therapies have become the favored practice in the fight against malaria due to resistance to two or more drugs used in combination will evolve more slowly. The preferred drugs in the eradication of malaria and are frequently used in combination therapies are artemisinin derivatives, which have rapid action, well tolerated and highly effective but they are expensive [32].

However, despite these efforts, there is a high rate of recrudescence associated with artemisinin monotherapy and it is common even when ACT is used. Resistance to artemisinin derivatives, the most potent antimalarial drugs currently used, and multidrug resistance to ACT have been reported and emerged in Southeast Asia and threaten to spread to Africa. This raises serious concerns and accelerating problem accounts for substantial number of excess deaths, especially among children. The loss of artemisinin derivatives would have devastating consequences on people's health in malaria-endemic countries and threaten the recent progress in malaria control achieved in many countries [3, 33].

1.3.2. Antimalarial Drug Toxicities

During management of infectious diseases such as malaria, adverse drug reactions (ADRs) contribute to ill-health or life-threatening outcomes of therapy. Household costs associated with ADR treatment are high and potentially catastrophic [34].

Antimalarial drug toxicity is viewed differently depending upon whether the clinical indication is for malaria treatment or prophylaxis. A greater risk of ADRs to antimalarial drugs is inevitable in the treatment of *P. falciparum* malaria. Therefore, drug toxicity must be acceptable to patients and cause less harm than the disease itself [35, 36].

Serious toxicity caused by antimalarial drugs include neuromyopathy, retinopathy, generalized convulsion and idiosyncratic reactions by chloroquine; cutaneous and liver toxicities as well as hypersensitivity and hematologic reactions by Fansidar (sulfadoxine-pyrimethamine); skin and neuropsychiatric toxicity by mefloquine; ocular, auditory, cardiac, cutaneous, hepatic and hematological toxicities and hypoglycemia by quinine; cardiotoxicity by halofantrine; anaphylactic reaction by Malarone (atovaquone-proguanil); hemolysis by primaquine; and allergic reactions by artemisinin derivatives and others [36, 37].

1.3.3. Plants as Sources of Antimalarial Agents

Globally higher plant species are estimated from 250,000 to 500,000. Relatively small percentages (1 to 10%) of these are used as foods by both humans and other animal species. From 14 to 28% of these have been used medicinally, but only 15% of them have been explored chemically [38, 39].

Approximately 6,500 higher plant species distributed in about 245 families are found in Ethiopia. From these about one-third of the families have been used in traditional medicinal practices. Ethiopia is one of the six plant biodiversity rich countries of Africa. About 1,000 medicinal plant species are identified; however, many others are not yet known [38].

The demand for medicinal plants is increasing in both developing and developed countries. About 80% of the world's population especially in developing countries still uses plant-based traditional medicine for their primary healthcare demands. Plant-based traditional medicines

are often termed complementary or alternative medicine and their use has steadily increased nowadays in developed countries as well. Also more than 30% of modern medicines are directly or indirectly derived from such plants [40].

About 70% of human and 90% of livestock population depend on such plants for their primary healthcare demands in Ethiopia. Ethiopian plants have shown very effective medicinal value for different ailments including malaria [41].

For more than 3000 years, traditional medicines have been used for the treatment of various diseases in general and malaria fever in particular. Medicinal plants form the principal component of traditional medicine [29]. Compounds containing novel structures from such natural origin represent a major source for the discovery and development of new drugs including antimalarials. Especially African flora such as Ethiopian's holds an enormous potential for the development of phytomedicines. The advantage of natural compounds for the development of drugs derives from their innate affinity for biological receptors [40, 42].

In the face of current resistance problems, medicinal plants could be a potential source of new, effective and affordable antimalarial agents. However, most of these plants, represent a virtually unlimited reservoir of molecules, are not explored chemically and can constitute lead molecules for development of new antimalarial drugs. Therefore, antimalarial drug discovery from natural sources is currently more targeted because histories proved that plants are rich sources of antimalarial phytochemicals [26, 40].

Herbal medicine is as old as the history of mankind. It is the surest source of effective antimalarials. Quinine and artemisinins, two main classes of antimalarial drugs, currently in use have been provided by plants traditionally used for malaria. Although it was incomplete, a database of ethnobotanical studies showed that over 1,277 plant species from 160 families have been used for the treatment of malaria globally. Nature particularly plants used in traditional medicine are a potential source of new antimalarial drugs as they contain molecules with a great variety of structures and pharmacological activities. Therefore, plant-derived antimalarials have made and continue to make a great contribution to antimalarial chemotherapy [25, 43, 44].

1.3.4. Antimalarial Evaluation of Medicinal Plants

About 74% of pharmacologically-active plant derived compounds were discovered after following up on ethnomedical use of the plant. Hence, compared to the random screening approach, the search of new and effective antimalarial agents and candidates based on ethnobotanical and ethnopharmacological approach has been proved to be more predictive. Therefore, ethnopharmacological use of plants can be a basis for phytochemical and phyto-pharmacological investigation. Although therapies involving herbal medicinal agents have shown promising potential, many of them remain untested scientifically and hence, their efficacy and safety is unknown [39, 44, 45].

Most of the presently used antimalarial drugs have been developed from information and exploration of medicinal plants. Extracts prepared from such traditionally claimed medicinal plants should, therefore, be evaluated for efficacy and safety by *in vitro* and *in vivo* standard tests. Plants widely used for treatment of malaria by traditional healers are significantly more active *in vitro* and/or *in vivo* against *Plasmodium* species than plants which are not widely used, or not used at all [46].

New antimalarial agents such as plant extracts can be tested by several methods. *In vitro* against *P. falciparum* and *in vivo* against animal models' plasmodia are the most commonly used ones. Avian, non-human primates and rodents are animal models for such *in vivo* studies. However, the rodent malaria model in mice is the most extensively used, and ideal for the primary *in vivo* test. Several rodent parasites can induce malaria in rodents but *P. berghei* is the most commonly used one for the study of malaria chemotherapy [14, 46, 47].

Rodent models have been validated through the identification of several conventional antimalarial drugs such as mefloquine, halofantrine and artemisinin derivatives. They have proven predictions in the treatment outcomes for human infections even though species differences exist. They used to produce the disease with a natural history similar to human malaria such as high parasitaemia, convulsions, respiratory distress and finally death. Hence, these models play a significant role in antimalarial drug discovery and development [46]. Conducting preliminary pharmacological screening study of crude extracts in such models is also logical and cost-effective [14].

1.3.5. Botanical Description of the Experimental Plant

Brassica nigra is grouped under the Family of *Brassicaceae* and Genus of *Brassica L.* [48].

1.3.5.1. The Family *Brassicaceae*

The family includes herbs, sometimes shrubs. It contains about 370 genera and 3,500 species with greatest variety in the Mediterranean area, west and central Asia and parts of North America. About 61 species in 23 genera are found in Ethiopia and Eritrea. The family includes several important oil and vegetables cultivated at higher altitudes in Ethiopia, as well as a large number of weeds, some ornamentals and plants of medicinal importance [48].

1.3.5.2. The Genus *Brassica L.*

The genus *Brassica* includes annual to perennial herbs. It is represented by about 150 species that are cultivated throughout the world as oilseed crops and/or vegetables, principally in the Mediterranean region and five of them including *Brassica nigra* are found in the flora of Ethiopia and Eritrea [48].

1.3.5.3. The *Brassica nigra (L.) Koch.*

Brassica nigra is commonly called black mustard. It is native to the southern Mediterranean region of Europe. It is an annual, erect, aromatic, up to 100 cm tall with freely and widely branching, and glabrate weedy herb. Leaves petiole; lower ones slender petiole, deeply pinnatifid, irregularly serrate with a large terminal lobe and 1 to 3 pairs of smaller side lobes; upper leaves undivided, practically entire. Sepals are 3 to 4 mm long. Petals 6 to 9 mm long, bright yellow and clawed. Fruits erect, appressed to the stem; beak slender, 2 to 4 mm long; valves keeled by mid-nerve; pedicels 3 to 6 mm long, erect. Seeds 1.4 to 1.6 mm, sub-globose, dark brown, finely reticulate. Its seeds grow in long, slender pods. Each pod contains 10 to 12 seeds, shown in Figure 2 [48].

Figure 2. Pictures of *Brassica nigra* and its seeds [49].

Brassica nigra is a weed in cultivated fields and waste places. It is also cultivated for its seeds. It grows at 1600 to 2450 m altitude. In Ethiopia, it is called “Senafitch” in Amharic. It is used to make a condiment used particularly during fasting seasons. It is commonly found in Tigray, Gondar, Sidamo, Arsi, and Harerge regions and most likely throughout the northern highlands in Ethiopia [48].

1.3.5.3.1.Traditional Medicinal Use of *Brassica nigra*

Brassica nigra is used throughout the world for various aspects such as food, vegetable, medicinal and industrial products. The medicinal effect is mainly confined to its seeds. The seeds have been used as tonic, digestive, emetic, appetite stimulant, laxatives, antiseptics and for carcinoma and tumors [50], liver and spleen indurations, abscesses, colds, headaches, rheumatism, edema, neuralgia, alopecia, epilepsy, snakebite, toothache, hiccup, pneumonia [51] and malaria [52, 53]. Its seeds are powdered and a paste have been made with water and then eaten with “Enjera” especially for the prevention as well as the treatment of malaria in Ethiopia [52].

1.3.5.3.2. Pharmacologic Activities of *Brassica nigra*

Anthelminthic in earth worm [54], antibacterial *in vitro* [55], larvicide in filaria mosquito larvae [56], anticholinesterase *in vitro* [57], anti-arthritic in rats [58], hypoglycemic and antidiabetic in rats [59], and antiepileptic and antioxidant in mice [60] are the reported pharmacologic activities of the extract of the seeds of *Brassica nigra*.

1.3.5.3.3. Phytoconstituents of *Brassica nigra*

The nutritional ingredients found in the seeds of *Brassica nigra* include proteins, carbohydrates, fibre, calcium, phosphorous, β-carotene, thiamine, riboflavin and niacin. Palmitic, stearic, oleic, linoleic, linolenic, and eicosenic acids are its fatty acid compositions [51]. Alkaloids, flavonoids, glycosides, phenols, sterols, tannins and terpenoids [55, 58, 61-63] are secondary metabolites reportedly detected by preliminary phytochemical screening of its seed extract.

Many classes of phytochemicals are involved in antiplasmodial activity from different plant extracts although the most significant and diverse bio-potency has been observed in alkaloids, quassinoids and terpenoids [45]. Quinine, oldest and most important antimalarial drugs, belongs to alkaloids. More than 100 alkaloids from higher plants were reported to exhibit considerable antimalarial activity; some were more potent than chloroquine. In addition, artemisinin, the most effective current antimalarial drug belongs to terpenoids [25].

1.3.5.3.4. Acute Toxicity of *Brassica nigra*

At different dose levels or even up to the limit dose of 2,000 mg/kg by oral administration of methanolic crude extract of the seeds of *Brassica nigra* to different groups of mice was found to be safe as reported by Uppala et al [63]. It did not produce any mortality or toxic manifestations up to that limit dose. Therefore, this safety data was used for the selection of the three dose levels (100, 200 and 400 mg/kg) of the extract of *Brassica nigra* for the current antimalarial activity evaluation in mice.

1.4. Significance of the Study

Currently new highly-effective antimalarial drug candidates, based on new mechanisms of action or with new structures, are urgently needed to overcome the problem of rapid emergence of resistance and achieve long-term clinical efficacy [39]. Additionally, unavailability and unaffordability of antimalarial drugs, the lack of an effective vaccine together with high toxicity and low therapeutic margin of existing antimalarial drugs emphasize the urgent need of well tolerated and affordable antimalarial drugs from traditionally claimed medicinal plants used in traditional medicine such as *Brassica nigra*.

The efficacy and safety of most medicinal plants in traditional medicine are not confirmed and their active constituents are not identified [27]. Hence, to establish a reliable quality control measure and to safe guard the health of the consumers, the herbal products must be augmented by pharmacological studies and scientific authentication of such products including *B. nigra*.

In addition, the seed extract of *Brassica nigra* showed potent antiepileptic activity [63] which might be important for the management of cerebral malaria which is associated with protracted and/or febrile convulsions. This activity of the plant might be used for the management of malaria related complications and hence conducting of this study on the antimalarial activity of the plant will be justified.

The plant endowed with various biologically active phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, and others, the various studies reported [55, 58, 61-63]. These chemicals have intrinsically antiplasmodial activity [44] and hence *in vivo* antimalarial evaluation of the current plant is relevant.

Brassica nigra is among the one traditionally used plant for the treatment of malaria in Ethiopia and elsewhere [52, 53]. However, its antimalarial activity is not scientifically reported in literature. Hence, it was considered practical to investigate its antimalarial activity in rodent malarial model to support or deject its folk use in the treatment of malaria.

2. OBJECTIVES

2.1. General Objective

- To evaluate the antimalarial activity of crude 80% methanolic extract of the seeds of *Brassica nigra* against *Plasmodium berghei* infection in mice

2.2. Specific Objectives

- To determine the chemosuppressive effect of the seed extract of *Brassica nigra* against *Plasmodium berghei* infection in mice
- To determine the chemoprophylactic effect of the seed extract of *Brassica nigra* against *Plasmodium berghei* infection in mice
- To evaluate the effect of the seed extract of *Brassica nigra* on the body weights and survival times of *Plasmodium berghei* infected mice

3. HYPOTHESIS

- ☞ The methanolic crude extract of the seeds of *Brassica nigra* possesses antimalarial activity.

4. MATERIALS AND METHODS

4.1. Materials

4.1.1. Collection of the Plant Materials

The seeds of fresh *Brassica nigra* were collected from the farmers' farmland in November 2014, around Gondar town, 728 km from Addis Ababa, Northwest Ethiopia. The plant's leaf was identified and authenticated and a voucher specimen (with a voucher number of AB05) was deposited at National Herbarium, Addis Ababa University, Ethiopia for future reference.

4.1.2. Experimental Animals

Fifty Swiss albino mice, weighing 25 to 32 gm and 6 to 8 weeks old, of either sex of inbred at the Animal House of the Department of Pharmacology, University of Gondar were used. They were housed in plastic cages with softwood shavings and chips as beddings. They had free access to pellet diet and clean drinking water. All mice were acclimatized to the working environment one week before the beginning of the experiment [64].

4.1.3. Parasites

In vivo evaluations of antimalarial activity begin with the use of the rodent malaria parasites since *Plasmodium* species that cause human malaria are basically unable to infect non-primate animal models (except in immunocompromised mice). Of the rodent malaria parasites, *P. berghei*, *P. yoelli*, *P. chabaudi* and *P. vinckei*, the most extensively used for initial antimalarial drug evaluation is the *P. bergei* because it produces lethal infection in mice that mimic human malaria. It also infects both mature and immature RBCs [47, 65].

Hence, chloroquine sensitive *P. berghei* (ANKA strain) was used for induction of malaria in experimental mice. Mice previously infected with *P. berghei* were used as donor. The donor *P. berghei* infected mice were obtained from Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia. The parasites were subsequently maintained in the laboratory by serial passage of blood from donor infected mice to naive one via intra-peritoneal (IP) route on weekly basis [47].

4.1.4. Chemicals and Instruments

Chloroquine (Addis Pharmaceuticals Factory PLC, Ethiopia), methanol (Okhla Industrial, India), Giemsa (Sciencelab, USA), trisodium citrate (Deluxe Scientific surgico PLC, India) and normal saline (Addis Pharmaceuticals Factory PLC, Ethiopia) were used. Whatmann filter paper number 1 (Whatman, England), dry oven (250±10% volts, France), electrical balance (ADP 720L, Adam Equipment Co. Ltd), microscopic slides (Citoplus, China), microscope (Binacular Olympus, Japan), mortar and pestle, oral gavage and mice cage were used. All chemicals and reagents were analytically graded and procured from certified suppliers.

4.2. Methods

4.2.1. Preparation of Plant Materials

The seeds of *Brassica nigra* were cleaned and air-dried in the shade at room temperature. The dried seeds were coarsely powdered using mortar and pestle. Then the powdered plant materials were stored in a plastic container and they were kept at room temperature until extraction.

4.2.2. Extraction of Plant Materials

The powdered samples of seeds of *B. nigra* (325 gm) were weighed by sensitive electrical balance. They were extracted by maceration with 80% methanol for three consecutive days with occasional stirring [26]. After three days, the mixture was filtered with Whatman filter paper number one. The residue was re-macerated twice for the same duration of days and then the mixture were filtered. The combined filtrates were dried by oven set below 40°C. The weight of the dried extract was measured to determine the percentage yield. Finally, the dried extract was then transferred into a vial and it was kept in a desiccator until further use. The extraction procedure was summarized in Figure 3.

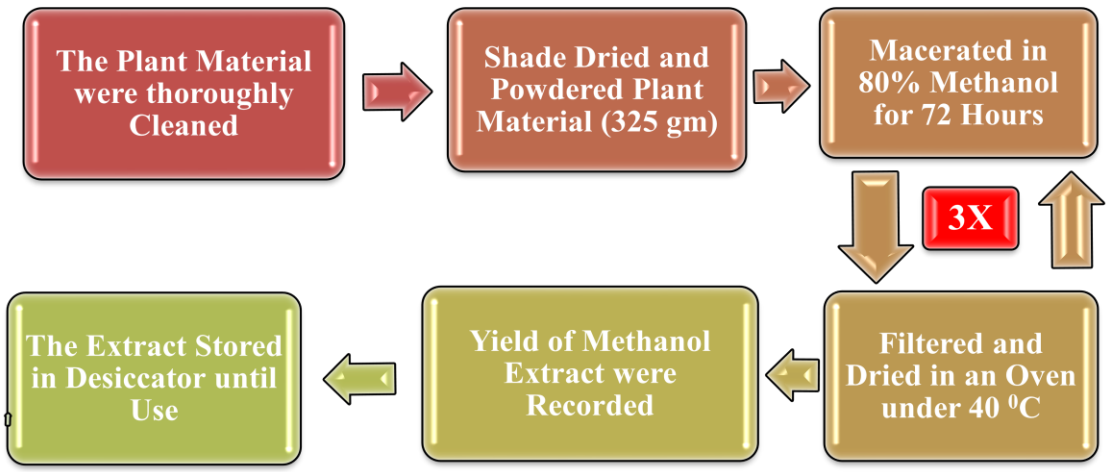

Figure 3. Schematic diagram of extraction procedure.

4.2.3. Study Design and Sampling Method

Experimental study design was used. Simple random sampling technique was employed for grouping of experimental animals and assignments of treatments.

4.2.4. Inoculum Preparation

The parasitemia of the previously *P. berghei* infected donor mouse were determined. The blood from these mice was collected via cardiac puncture with a rising parasitaemia of 30 to 35% into a test tube having 0.5% trisodium citrate which was added as anticoagulant. The blood was then diluted with normal saline (0.9%) to give 2 x 10^7 IRBCs in an injection volume of 0.2 ml. The amount of normal saline used in the dilution was determined by the level of parasitaemia (%) of the infected donor mice. If 1 ml of blood was removed then dilution was done with (X-1) ml of saline, where X was the percentage parasitaemia. Each mouse was then infected by injecting 0.2 ml of this diluted blood via IP which contained 2 x 10^7 IRBCs, which produces a steadily rising infection in mice [47, 66].

4.2.5. Grouping and Dosing of Animals

For each model (suppressive and prophylactic), twenty five mice were grouped into five groups of five mice each. Group I mice were treated with the vehicle (distilled water, 10 ml/kg, served as negative control), group II, III and IV mice were treated with 100, 200, and

400 mg/kg of crude extract, respectively and the last group (group V) mice were treated with the standard antimalarial drug (chloroquine, 10 mg/kg, served as positive control).

As people traditionally use the plant material for treatment and prevention of malaria orally [52], each treatment (the vehicle, the standard drug and the extract) was administered through intragastric route using standard tube (oral gavage) to ensure safe ingestion of the preparations.

4.2.6. Pharmacological Screening Tests

4.2.6.1. Four-Day Suppressive Test

Plasmodium berghei four-day suppressive test, the most widely used preliminary test, in which the efficacy of four daily doses of new compounds is measured by comparison of blood parasitaemia and survival times of treated and untreated mice. The test provides a preclinical indication of potential bioactivity of the extract [46, 47]. The chemosuppressive test was done by using a standard four-day suppressive test against *P. berghei* infected mice as described by Fidock et al [47]. After standard parasite inoculation, 25 mice were randomly divided into five groups with five mice each as above mentioned. Treatment was started three hours post-infection for each group accordingly and then continued for three consecutive days (i.e., from D_0 to D_3). On the 5^{th} day (D_4), thin blood film was made from the tail of each mouse on a microscopic slide.

4.2.6.2. Prophylactic Test

Compounds identified as being active in four-day *in vivo* assays can subsequently be further examined through the use of several secondary tests in mice from which prophylactic test are one of them. Evaluation of prophylactic potential of the extract was also done by methods described by Fidock et al [47]. Twenty five mice were randomly grouped into five groups as mentioned above accordingly and then treated for four consecutive days (D_0 to D_3). On the fifth day (D_4), a standard inoculum (about 2 x 10^7 *P. berghei* IRBCs, 0.2 ml) was administered by IP to each mouse. After 72 hours of parasite inoculation (D_7), thin blood smears were prepared from tail of each mouse on a microscopic slide.

4.2.6.3. Peripheral Blood Smear Preparation

For both models (suppressive and prophylactic), thin smear of blood were made from the tail of each mouse on the fifth day (D_4) and eighth day (D_7), respectively. The smears were applied on microscopic slides and the blood was drawn evenly across a second slide to make a thin blood film and allowed to dry at room temperature. Then they were fixed with absolute methanol and stained with 10% Giemsa stain at pH 7.2 for 15 min. Each stained slide for each mouse was examined under microscope.

4.2.6.4. Parasitemia Determination

The parasitaemia level was determined by counting the number of parasitized erythrocytes in random fields of the microscope. The smears were counted and read by a laboratory technician to make the reader blind to the category. Average parasitaemia and percent parasitemia suppression were calculated using the following formula [47, 65].

$$\% \text{ Parasitaemia} = \frac{\text{Number of Infected RBCs}}{\text{Total Number of RBCs}} \times 100$$

$$\% \text{ Suppression} = \frac{(\text{Mean Parasitemia of Negative Control} - \text{Mean Parasitemia of Treated Group})}{\text{Mean Parasitemia of Negative Control}} \times 100$$

4.2.6.5. Determination of Mean Survival Time

Mean survival time (MST) is another parameter that is commonly used to evaluate the efficacy of antimalarial plant extracts [25]. An extract that results in survival time greater than that of infected non-treated mice was considered as active. Death occurring before day five of infected and treated mice was regarded as toxic death. Mortality was monitored daily and the number of the days from the time of inoculation of the parasite up to death was recorded for each mouse in the treatment and control groups throughout the follow-up period.

The survival time for each mouse was recorded after the treatment periods and the MST was calculated for each group by using the following formula.

$$\text{MST} = \frac{\text{Sum of Survival Time of All Mice in a Group (days)}}{\text{Total Number of Mice in That Group}}$$

4.2.6.6. Body Weight Determination

Similarly, body weight loss is one feature of rodent malaria infections [65]. Body weight of each mouse was measured to determine the effectiveness of the extract. The body weight of each mouse in all groups was taken before infection (D_0) and treatment (D_1) and after treatment (D_4) and inoculation (D_7), in suppressive and prophylactic tests, respectively. The weight of each mouse was measured using sensitive electrical balance. Then, the average body weight change (%) of extract treated groups was compared with the control groups. The average body weight change (%) of each treatment group was calculated by the following formula.

$$\text{Average Wt Change (\%)} = \frac{\text{Average } D_4 \text{ (or } D_7\text{) Wt of a group} - \text{Average } D_0 \text{ (or } D_1\text{) Wt of that group}}{\text{Average } D_0 \text{ (or } D_1\text{) Wt of that group}} \times 100$$

4.2.7. Statistical Analysis

Results of the study were expressed as mean ± SEM (standard error of mean) for each treatment group. Data on parasitemia, body weight changes and survival times were analyzed using windows SPSS version 16.0. One-way ANOVA was used to analyze differences among groups. Subgroup analysis was conducted using Turkey's HSD *post-hoc* test. Body weights, before infection and after treatment, were compared by two-tailed paired t-test. The difference was considered statistically significant if P-value < 0.05 at 95% confidence level.

4.2.8. Data Quality Control

The quality of data was assured by using randomization during grouping of experimental animals and assignments of treatments. Codes were used for all microscopic slides. Parasitized RBCs were counted blindly by a laboratory technician.

4.2.9. Ethical Consideration

During experimental procedures, experimental animals were handled and cared according to the internationally accepted laboratory animals' use, care and welfare guideline [64]. Ethical clearance was requested and approved by the Department of Pharmacology, School of Pharmacy, University of Gondar.

5. RESULTS

5.1. Percentage Yield of the Plant Material

The percentage yield of the dry matter of the 80% methanolic crude extract of the seeds of *Brassica nigra* was found 9.85% w/w. Its actual yield was 32 gm. It was red in its colour. Hygroscopic powder was formed after drying.

5.2. Four-day Chemosuppressive Antimalarial Activity of the Extract

The methanolic crude extract of the seeds of *Brassica nigra* showed chemosuppressive activity against *Plasmodium berghei* in mice. At three dose levels (100, 200 and 400 mg/kg body weight) of the extract, there were 21.88, 50.00 and 53.13% chemosuppressions, respectively, shown in Table 1.

Table 1: Suppressive activity of methanolic crude extract of the seeds of *Brassica nigra* against *Plasmodium berghei* infection in mice (values are expressed as Mean ± SEM; n = 5).

Treatments	Doses	% Parasitemia	% Chemosuppression
Distilled water	10 ml/kg	25.60±2.40	0.00
Brassica nigra seed extract	100 mg/kg	20.00±2.53	21.88^{b3}
	200 mg/kg	12.80±0.80	$50.00^{a2,b2}$
	400 mg/kg	12.00±2.83	$53.13^{a2,b1}$
Chloroquine	10 mg/kg	1.40±0.75	94.53^{a3}

Where a=as compared to negative control; b=as compared to positive control; 1=P < 0.05; 2=P < 0.01; and 3=P < 0.001.

Even though there were differences in parasitemia suppression among extract treated groups, they were statistically insignificant ($P > 0.05$). The extract exhibited a dose-dependent activity, as the dose increased also antiplasmodial activity increased significantly. The chemosuppression caused by chloroquine was statistically significant ($P < 0.05$) as compared to other treatment groups.

5.3. Chemoprophylactic Antimalarial Activity of the Extract

The extract of *Brassica nigra* also showed chemoprophylactic activity against *Plasmodium berghei* in mice. At three dose levels (100, 200 and 400 mg/kg body weight) of the extract, there were 17.42, 21.21 and 53.79% suppressions, respectively, shown in Table 2.

Table 2: Prophylactic activity of methanolic crude extract of the seeds of *Brassica nigra* against *Plasmodium berghei* infection in mice (values are expressed as Mean ± SEM; n = 5).

Treatments	Doses	% Parasitemia	% Chemosuppression
Distilled water	10 ml/kg	26.40±5.80	0.00
Brassica nigra seed extract	100 mg/kg	21.80±2.65	17.42^{b2}
	200 mg/kg	20.80±3.01	21.21^{b2}
	400 mg/kg	12.20±1.36	53.79^{a1}
Chloroquine	10 mg/kg	1.00±0.45	96.21^{a3}

Where a=as compared to negative control; b=as compared to positive control; 1=P < 0.05; 2=P < 0.01; and 3=P < 0.001.

There was no statistically significant (P > 0.05) chemoprophylactic differences among extract treated groups. As four-day suppressive test, the extract showed a dose-dependent chemosuppressive activity. Chemoprophylactic activity produced by chloroquine was also significant (P < 0.01) as compared to other treatment groups except 400 mg/kg treated ones.

5.4. Effect of Extract on Survival Times of Mice

In the four-day suppressive test, the extract treated groups lived longer than the corresponding negative control. However, there were no observed differences in survival times among extract treated groups. In case of chloroquine treated mice, all of them lived in the observation period (within 15 days). Chloroquine treated mice significantly (P < 0.001) lived longer than other treated groups, shown in Table 3.

Table 3: Effect of crude methanolic extract of the seeds of *B. nigra* on the mean survival times of *P. berghei* infected mice in both suppressive and prophylactic tests (values are expressed as Mean ± SEM; n = 5).

Tests	Treatments	Doses	Mean Survival Times (Days)
Suppressive	Distilled water	10 ml/kg	7.20±0.37
	Brassica nigra seed extract	100 mg/kg	7.60 ± 0.25^{b2}
		200 mg/kg	$9.00\pm0.45^{a1,b2}$
		400 mg/kg	$9.00\pm0.71^{a1,b2}$
	Chloroquine	10 mg/kg	15.00 ± 0.00^{a2}
Prophylactic	Distilled water	10 ml/kg	6.80±0.20
	Brassica nigra seed extract	100 mg/kg	7.00 ± 0.32^{b2}
		200 mg/kg	7.60 ± 0.25^{b2}
		400 mg/kg	7.80 ± 0.37^{b2}
	Chloroquine	10 mg/kg	14.60 ± 0.40^{a2}

Where a=as compared to negative control; b=as compared to positive control; 1=P < 0.05; and 2=P < 0.001.

In prophylactic test, the survival times did not prolonged statistically ($P > 0.05$) when compared to negative control. Chloroquine treated mice significantly ($P < 0.001$) lived longer than any other treated groups.

5.5. Effect of Extract on Body Weights of Mice

In the four-day suppressive test, all extract treated groups prevented in the body weight loss of mice when compared to negative control. However, there were no significant ($P > 0.05$) differences in weight gain among extract treated groups, shown in Table 4.

Table 4: Effect of crude methanolic extract of the seeds of *B. nigra* on the body weights of *P. berghei* infected mice in suppressive test (values are expressed as Mean ± SEM; n = 5).

Treatments	Doses	Body Weight (g)		
		D_0-Weight	D_4-Weight	Wt Change (%)
Distilled water	10 ml/kg	29.64±0.34	29.30±0.32	-1.15
Brassica nigra seed extract	100 mg/kg	30.38±1.89	30.48±2.05	0.33
	200 mg/kg	26.68±1.57	26.92±1.57	0.90[a1]
	400 mg/kg	29.26±1.41	29.62±1.42	1.23[a1]
Chloroquine	10 mg/kg	28.18±1.64	28.58±1.65	1.42[a2]

Where a=as compared to negative control; 1=P < 0.01 and 2=P < 0.001.

In the prophylactic test, there was no statistically significant body weight change of extract treated groups when compared to negative control even though a weight gain was there. Positive control mice showed a significant (P < 0.05) body weight gain compared to negative control but insignificant (P > 0.05) as compared to extract treated groups, shown in Table 5.

Table 5: Effect of crude methanolic extract of the seeds of *B. nigra* on the body weights of *P. berghei* infected mice in prophylactic test (values are expressed as Mean ± SEM; n = 5).

Treatments	Doses	Body Weight (g)		
		D_1-Weight	D_7-Weight	Wt Change (%)
Distilled water	10 ml/kg	24.94±1.99	24.48±2.00	-1.84
Brassica nigra seed extract	100 mg/kg	26.80±0.99	27.00±0.94	0.75
	200 mg/kg	25.84±2.42	26.06±2.50	0.85
	400 mg/kg	28.10±1.56	28.42±1.68	1.14
Chloroquine	10 mg/kg	26.86±1.13	27.34±1.17	1.79[a1]

Where a=as compared to negative control and 1=P < 0.05.

6. DISCUSSION

The antimalarial activities of the crude methanolic extract of the seeds of *Brassica nigra,* used in traditional medicine in Ethiopia and elsewhere, against *Plasmodium berghei* infected mice in both four-day suppressive and prophylactic test models are reported.

The percentage suppression of parasitaemia of the extract treated groups were changed significantly from those in the negative control group showing that the extract has antimalarial activity supporting the folk use of the plant as antimalarial herb. A compound is considered as active when reduction in parasitemia is 30% or more [14, 67], which supports the findings of the current study.

The mice treated with 200 and 400 mg/kg of extract showed significant 50.00% and 53.13% chemosuppressions, respectively as compared to negative control. The chemosuppressive effects of the extract of the seeds of *Brassica nigra* in the current study were in agreement with other reports on medicinal plants for malaria such as *Agelanthus dodeneifolius* [68], *Echinops kebericho* [69], *Phytolacca dodecandra* [70] and *Sida rhombifolia* [71], which showed significant 48.02% and 50.50%; 29.46% and 57.29%; 50.93% and 55.24%; and 50.10% and 52.30% chemosuppressions, respectively with similar doses.

According to Deharo et al [72], the *in vivo* antimalarial activity of plant extract can be categorized as: if the extract showed 50% or more chemosuppression at 500, 250 and 100 mg/kg/day, then the *in vivo* antimalarial activity was considered as moderate, good and very good, respectively and when the suppression is higher than 50%, the activity is considered moderate at the dose of 1,000 mg/kg per day otherwise it is considered as inactive. Hence, the current studied plant was showed good four-day suppressive antimalarial activity.

The methanolic seed extract of *B. nigra* also showed a significant chemoprophylactic activity against residual infection of the parasite at the dose of 400 mg/kg which showed 53.79% suppression. The current finding was in agreement with other reports on medicinal plants for malaria such as *Agelanthus dodeneifolius* [68] and *Languas galangal* [73], which showed significant 56.00% and 52.00% chemosuppression, respectively. According to Deharo et al

[72] *in vivo* antimalarial activity classification, the methanolic crude extract of the seeds of *Brassica nigra* showed moderate chemoprophylactic antimalarial activity.

In both models (suppressive and prophylactic), chloroquine, the current antimalarial drug, was used as a reference drug [25, 47] and it showed parasitemia suppression near to undetectable levels of 94.53 and 96.21%, respectively. It leads to decreased parasitemia level and resultant recovery of severe malaria by reducing parasite nutrient intake as well as by interfering with parasite metabolic machinery involved with iron [73]. The effect of chloroquine on parasitaemia level in this study was in agreement with other studies on medicinal plants for malaria such as *Adhathoda schimperiana* [74], *Artemisia annua* [75], and *Annona senegalensis* [76] which were 97.80%, 90.48% and 96.20%, respectively.

The pharmacological (i.e., the antiplasmodial) activities of plants are due to the presence of bioactive secondary metabolites in the crude material [45]. Basically, different secondary metabolites, such as alkaloids [55, 62, 63], flavonoids [61-63], glycosides [55, 63], phenols, terpenoids and sterols [55] and tannins [61, 62], have been reported from the seed extract of *Brassica nigra*. These metabolites have antiplasmodial activities [42-45]. However, the active compound(s) known to give this observed antiplasmodial activity need to be identified.

Therefore, the antiplasmodial activity observed in this plant could have resulted from single or in synergetic action of the above metabolites [77]. The possible mechanisms of antiplasmodial activity might be through anti-oxidation and free radical scavenging [47], stimulating natural and adaptive defense mechanisms [76], intercalation in DNA, inhibition of protein synthesis and interference with the invasion of new erythrocytes by parasites or by other unknown mechanisms [68, 77].

Mean survival time is another parameter that is commonly used to evaluate the efficacy of antimalarial plant extracts. An extract that results in survival time greater than that of infected non-treated mice was considered as active [25]. The mice treated with 200 and 400 mg extract had significantly lived longer than negative control in four-day suppressive test. This might be due to the antiplasmodial activity of the extract. The current findings were in

agreement with studies done on medicinal plants for malaria such as *Dodonaea angustifolia* [78] and *Nigella sativa* [79].

Body weight loss is one manifestation of *Plasmodium berghei*-infected mice. This was due to the depressant action of the parasite on the appetite of the mice and the consequences of disturbed metabolic function and hypoglycaemia [66]. The antimalarial activity of the extract can also be assessed indirectly by observing the effect of the extract on body weight of mice [80]. Mice treated with 200 and 400 mg/kg of extract brought a significant increased in body weight when compared to negative control, hence, this might tell us the antiplasmodial activity of the extract.

The plant possesses other pharmacologic benefits which in part increased weight gain such as appetite stimulant activity [50] and nutritionally it is endowed with vitamin Bs such as B_1 (thiamine which maintains appetite and growth), B_2 (riboflavin which prevents skin lesions and weight loss) and B_3 (niacin which maintains the normal function of the nervous system and the gastrointestinal tract) [51]. This might in part lead to increased in body weight gain. Insignificant weight gain of mice was observed in prophylactic test. This might be the rapid hepatic clearance of the active component of the plant, which was given before infection at which the basal metabolic rate of the mice active, involved in weight gain. This finding was in agreement of the study on medicinal plant of *Faidherbia albida* [81].

7. CONCLUSION AND RECOMMENDATIONS

7.1. Conclusion

From this study, it can be concluded that the seeds of *Brassica nigra* exhibited good suppressive and moderate prophylactic activities against *Plasmodium berghei*. This plant could be the potential source of new and novel antimalarial leads and/or drugs for the treatment and prophylaxis of malaria. Therefore, this study supports further investigations on the active components of the plant.

7.2. Recommendations

Based on the present findings, the following suggestions for further studies are made on:

- *In vitro* investigation of the plant against human malaria parasites.
- Further pharmacological screening with bioassay guided chemical fractionations of the crude extract of the plant to isolate, identify and characterize the potential new antimalarial active compound(s).
- Chronic and cytotoxicity tests of the crude extract and isolated fractions.

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